# RESEARCH PROPOSAL

Herb-drug interaction study of Antidiabetic plant extract and common Oral Antidiabetic Drug

For Registration to the Degree of
Doctor of Philosophy
**IN THE FACULTY OF SCIENCES**

## IIS (deemed to be UNIVERSITY), JAIPUR


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**DISCIPLINE OF ZOOLOGY**

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## Introduction

India has been a land of *Vedas* and *Ayurveda*. The people have heavily relied on the herbal remedies to complement the conventional therapies for treatment of various chronic illnesses. One such disease is diabetes. In the year 2019, there were around 77 million patients for diabetes across the country (age group 20-79 years). The *International Diabetes Federation (IDF)* highlighted that by the year 2045, the prevalence of diabetes is expected to double (Sivaprasad et al., 2020).

The major portion of the population of the country is comprised of people residing in rural areas or people with low-socio economic background residing in urban areas. These people are not able to readily afford the costly conventional therapies used in diabetes. Hence, they depend on herbal supplements and folklore medicines. The prime reasons for the frequent use of these herbs can be their low cost, easy accessibility, fewer side effects and low rates of causing dependency among patients (Pandey et al., 2013).

When a herb is co-administerd with a drug by a patient, then it may have both pharmacokinetic (PK) and pharmacodynamic (PD) interactions on the latter (Kim et al., 2014). Phytochemically, the medicinal plants are known because of their repository of complex mixtures of bioactive compounds that can alter the absorption rate or the oxidative metabolism of the co-administered drug leading to PK interactions(Cho & Yoon, 2015). This occurs due to inhibition/induction of the intestinal and hepatic drug metabolizing enzymes (DMEs) like cytochromes (CYPs) as well as efflux and drug transporter (DT) proteins (like P-glycoprotein (P-gp)) (Meijerman et al., 2006). Whereas, in the PD interactions, the herb has a capacity to augment or inhibit the pharmacological activity of the co-administered drug through additive, synergistic or antagonistic actions (Wojcikowski et al., 2009).

The present study aims to study the effect of common antidiabetic plant species on the pharmacokinetics and pharmacodynamics of a commonly used oral antidiabetic drug class. At present we are considering three commonly found species in Rajasthan and in the pilot study screening of these species will be done to narrow down to one species based on its efficacy.

## Review of Literature

### Type II Diabetes: Overview

Sugar (or Glucose) is an important source of fuel for the body and Type II Diabetes is a chronic disease that affects the ways in which this fuel is metabolized. This type of diabetes is considered as a lifelong disease which prevents the body from using the insulin in the way it should otherwise use it. In this type, the body either resists the effects of insulin or it simply fails to produce enough insulin that is required to maintain the normal levels of glucose in the body (Olokoba et al., 2012). Earlier, this disease was considered as the *adult-onset* diabetes, but now it is becoming more common among children due to increasing cases of obesity among them. The doctors and researchers haven't suggested any cure for this disease till this date; however, they have suggested that improved lifestyles, healthy eating and daily workout routines can surely help the patients manage the disease easily. (Chatterjee et al., 2017). Apart from this, if the patients aren't able to manage this type of diabetes with diet and exercise, then they may also need specific medications or insulin therapy (Trikkalinou et al., 2017)

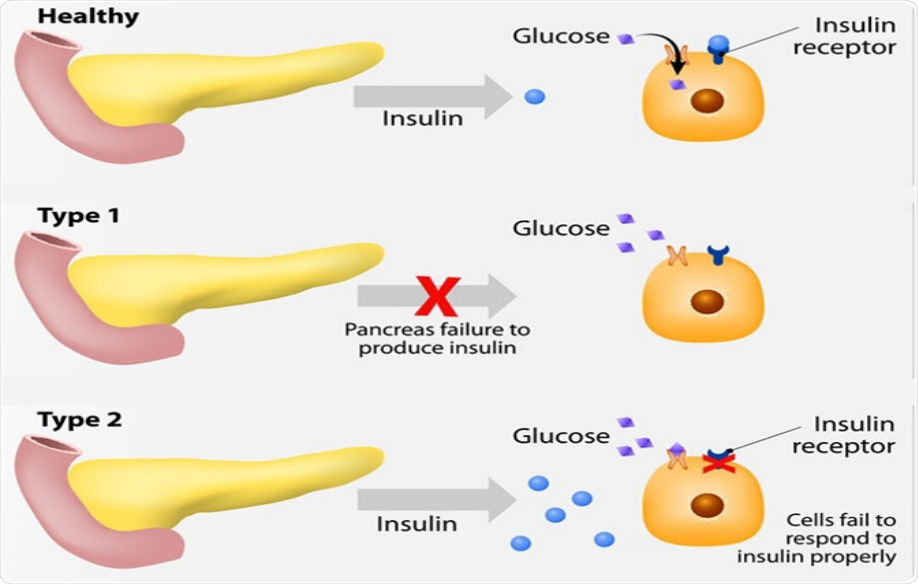



**Fig 1: Types of Diabetes** (Diabetes Mellitus Subtypes, 2019)

Signs and Symptoms of Type II Diabetes

The signs and symptoms of Type II Diabetes often develop quite slowly and the most common ones are increased thirst, fatigue and tiredness, slow healing of wounds and sores, frequent urination, increased hunger, blurred vision, darkened skin in the region of armpits and neck, etc. If this type of diabetes remains undetected for a longer duration, then unexpected weight loss also become common (Ramachandran, 2014).

Drug classes for Diabetes

In the case of Non-Insulin dependent Diabetes Mellitus (NIDDM) or Type II Diabetes, several classes of drugs are effective. As most of these drugs are administered orally, hence they are called *oral hypoglycemic agents* or *Oral antidiabetic drugs* (OADs) (Chaudhury et al., 2017; Rehani et al., 2019).

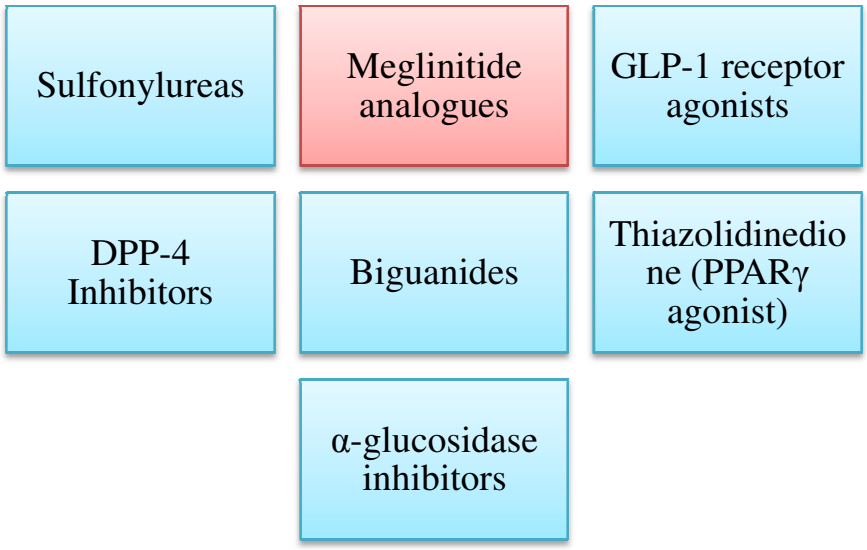



**Fig 2: Categories of Oral Anti-diabetic Drugs (OADs)**

The prime classes of drugs and their specific mode of actions are summarized below in *Table 1*.

*Table 1: Categories of OADs and their Mechanism of action.*

| S.No. | Drug Class | Mode of action (MOA) | Examples | References |
|---|---|---|---|---|
| 1. | Sulfonylureas | • Lowers glucose level by stimulating acute release of Insulin from β cells of the pancreas and decreasing its hepatic clearance.<br>• Specifically blocks $K_{ATP}$ channels on the β cell membrane. | Generation I: Tolbutamide<br><br>Generation II: Glibenclamide, Glimepiride, Glipizide, Gliclazide, Gliquidone | (American Diabetes Association, 2013; Sola et al., 2015) |
| 2. | Meglinitide Analogues (Glinides) | • Based on sulfonylurea moiety of glibenclamide.<br>• Same MOA as sulfonylurea. Binds to SUR1 receptor of β cells and stimulate insulin release by closure of $K_{ATP}$ channels. | Repaglinide, Nateglinide | (Guardado-Mendoza et al., 2013; Pakkir Maideen et al., 2018) |
| 3. | Glucagon-like | • Inhibits glucagon production from | Exenatide, | (Burcelin & |

| | | | | |
|---|---|---|---|---|
| | peptide-1 (GLP-1) receptor agonists | pancreatic α cells and delays gastric emptying.<br>• Decreases β cell apoptosis and promote their proliferation | Lixisenatide, Liraglutide, Albiglutide, Dulaglutid, Semaglutide | Gourdy, 2017; Hunt et al., 2019) |
| 4. | Dipeptidyl peptidase-4 (DPP-4) Inhibitors | • Increases incretin levels (GLP-1, GIP) which inhibit glucagon release. | Sitagliptin, Vildagliptin, Saxagliptin | (Thornberry & Gallwitz, 2009; Vella, 2012) |
| 5. | Biguanide | • Prevents liver to convert fats and AAs into glucose.<br>• Activate the enzyme AMPKwhich helps the cells to respond more effectively to insulin. | Metformin | (Rena et al., 2017; Song, 2016) |
| 6. | Thiazolidine-dione (PPARγ agonist) | • Tends to reverse insulin resistance by enhancing GLUT4 expression and translocation.<br>• Improves entry of glucose into muscles and fats | Pioglitazone, Rosiglitazone | (Cariou et al., 2012) |

*GIP: glucose-dependent insulinotropic peptide

AAs: Amino acids

AMPK: 5' adenosine monophosphate-activated protein kinase

PPARγ: Peroxisome proliferator-activated receptor-γ

*Meglinitide Analogues (Glinides)*

The meglitinide analogues ("glinides") are a class of oral antidiabetic agents that increases insulin secretion in the pancreas. The properties of this class of drug suggest that they have the potential to produce a rapid, short-lived insulin output (Black et al., 2007). Two analogues are currently available for clinical use: repaglinide and Nateglinide (Landgraf, 2000). Meglitinide analogues belong to a new family of insulin secretagogues which stimulate insulin release by inhibiting ATP-sensitive potassium channels of the β-cell membrane via binding to a receptor distinct from that of sulphonylureas. Repaglinide and nateglinide are absorbed rapidly, stimulate insulin release within a few minutes, are rapidly metabolized in the liver and are mainly excreted in the bile. Therefore, following preprandial administration of these drugs, insulin is more readily available during and just after the meal. This leads to a significant reduction in postprandial hyperglycemia without the danger of hypoglycemia between meals (Blicklé, 2006; Dornhorst, 2001).

*Mechanism of Action (MOA)*

A rise in plasma glucose levels associated with a meal results in increased glucose uptake, followed by intracellular metabolism resulting in an increase in intracellular ATP. A rise in

intracellular ATP inhibits ATP-sensitive $K^+$ channels $K^+$ channel inhibition results in membrane depolarization, with a resulting activation of depolarization-activated $Ca^{2+}$ channels. A rise in intracellular $Ca^{2+}$ promotes fusion of vesicles containing insulin with the cell membrane, and subsequent release of insulin. Sulfonylureas and glinides bind to a site on the sulfonylurea receptor (SUR1) channel subunit, which inhibits these channels. ATP binds to a different nucleotide-binding domain (NBD) on the cytoplasmic side. Binding of either ATP or these drugs to their sites of action produces a conformational change that inhibits $K^+$ pore permeability (Feingold, 2000; Landgraf, 2000). Glinides bind with lower affinity than sulfonylureas, and as a result have a much shorter duration of action. To be effective, glinides have to be given with each meal.

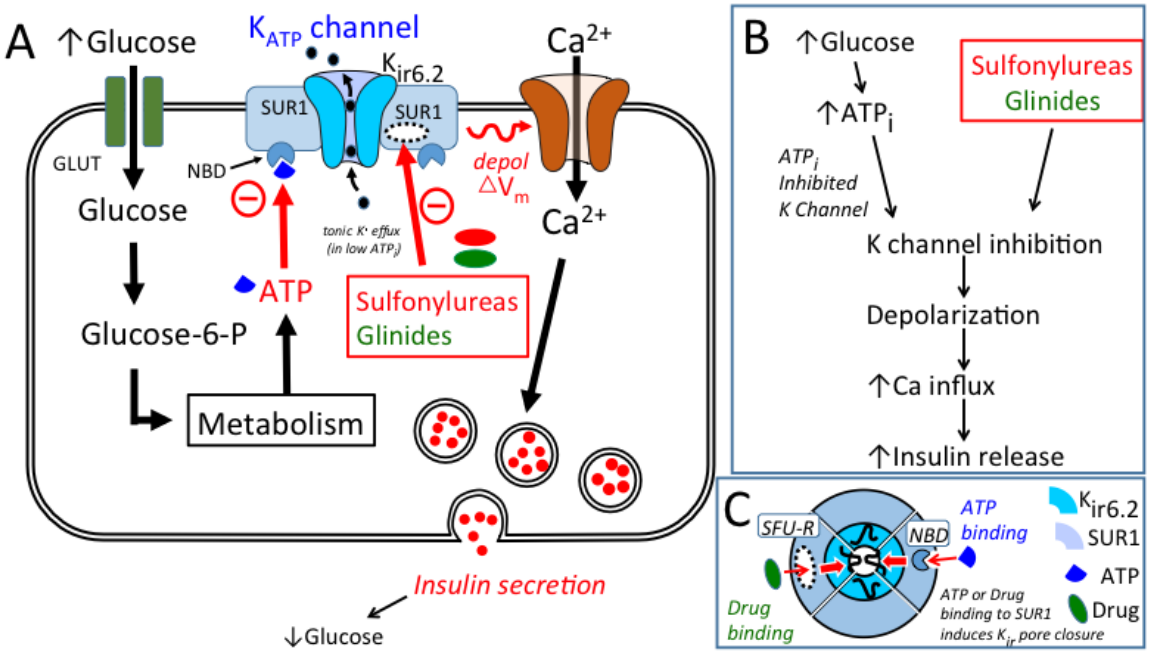



**Fig 3: Mechanism of Action of Glinides**

**Rajasthan: A land of anti-diabetic herbs**

With a land of around 342,239 $km^2$, Rajasthan has a rich biodiversity having a number of plants with great medicinal values. Around 75% of the total population lives in the village and utilizes local medicines for common and chronic ailments (Mohan et al., 2017). The soils of this state's desert plains are loamy sand and the eastern parts even have the alluvial soil which supports good forest and agricultural crops. The ICMR conducted a cross sectional survey on the people of India, named ICMR-INDIAB (INdia- DIABetes) and its phase III covered the state of Rajasthan. Below are the specific findings of the survey (in relation to Rajasthan):

- 5.7% of the state's population is suffering from diabetes (Chacko, 2019).

- No. of cases with pre-diabetes have increased faster than those with diabetes (Anjana et al., 2011).
- Prevalence of pre-diabetes is at 15.2% in the state (same as that in 15 more developed states) (Iqbal, 2019).
- Out of the total population of the state, 75.13% resides in rural and semi urban areas. These people do not have access to diabetes centers or clinics providing comprehensive care. Hence, they rely on traditional herbs and local treatments (Anjana et al., 2017).

*Commonly used antidiabetic species in Rajasthan*

For the pilot study of this research, there has been a consideration of three common antidiabetic species of Rajasthan, namely: *Acacia nilotica, Commiphora wightii* and *Cyamopis tetragonoloba*.

***Acacia nilotica (Babul)***

***Cyamopsis tetragonoloba (Gwar)***

***Commiphora wightii (Guggul)***

*Table 2: Common anti-diabetic species found in Rajasthan*

| Plant | Location | Part/s showing antidiabetic properties | Phytoconstituents | Fruiting/ Flowering season | References |
|---|---|---|---|---|---|
| ***Acacia nilotica (Babul)*** | Northern Rajasthan, particularly in the districts of Jaipur, Bharatpur, Alwar, Sawai Madhopur, Dausa, Kota and Bundi. | Leaves, bark, Flowers | Ellagic acid, kaempferol, quercetin, gallic acid, catechin, epicatechin (EC), epicatechin-3-*O*-gallate (ECG), epigallocatechin-3-*O*-gallate (EGCG) | Fruits: April and May | (Meena et al., 2006; Shekar et al., 2015) |
| ***Comiphora wightii (Guggul)*** | Southern part (Udaipur, Banswara, Dungarpur, Pratapgarh, Chittorgarh) | Gum, leaves, flowers | Guggulsterol-I, -II & -III, gugullipid, gum resin, myricyl alc. and β-sitosterol, gallic acid, quercetin, guggulsterone | Flowers-November-July (Max-April-July) Fruits: April-July | (Sarup et al., 2015; Shah et al., 2012) |
| ***Cyamopis tetragonoloba (Gwar)*** | Bikaner, Ganganagar, Hanumanghar and Churu. Western belt of Jaisalmer, Barmer and Jodhpur | Leaves, Seeds, beans | Caffeic acid, gallic acid, gentisic acid, p-coumaric acid, Quercetin, daidzein, kaempferol, myricetin- ellegic acid | Flowers and fruits: November - January | (Asati & Sharma, 2019; Overbeeke et al., 1990) |

**Published work (up to date) on antidiabetic activity of the selected species**

*Table 3: Reported literature on antidiabetic activity of Acacia nilotica (2011-2020)*

| S.No. | Title | Year of publication | Reference |
|---|---|---|---|
| 1. | *Acacia nilotica* leaf improves insulin resistance and hyperglycemia associated acute hepatic injury and nephrotoxicity by improving systemic antioxidant status in diabetic mice | 2018 | (Saha et al., 2018) |
| 2. | Hypoglycemic effects of *Acacia nilotica* in type II diabetes: a research proposal | 2017 | (Roozbeh et al., 2017) |
| 3. | Duration effect of *Acacia nilotica* leaves extract and glibenclamide as hypolipidaemic and hypoglycaemic activity in alloxan induced diabetic rats | 2015 | (Asad et al., 2015) |
| 4. | *In vitro* antioxidant and antidiabetic activities of biomodified lignin from *Acacia nilotica* wood | 2015 | (Barapatre et al., 2015) |
| 5. | Effect of *Acacia nilotica* fruit extract on serum glucose and lipid concentrations in alloxan-induced diabetic rats | 2013 | (Abuelgassim, 2013) |
| 6. | Optimized mixture of hops rho iso-alpha acids-rich extract and acacia proanthocyanidins-rich extract reduces insulin resistance in 3T3-L1 adipocytes and improves glucose and insulin control in db/db mice | 2012 | (Tripp et al., 2012) |
| 7. | Therapeutic effect of *Acacia nilotica* pods extract on streptozotocin induced diabetic nephropathy in rats | 2012 | (Omara et al., 2012) |
| 8. | *Acacia nilotica* leave extract and glyburide: comparison of fasting blood glucose, serum insulin, beta-thromboglubulin levels and platelet aggregation in streptozotocin induced diabetic rats | 2011 | (Asad et al., 2011) |
| 9. | Effect of *Acacia nilotica* leaves extract on hyperglycaemia, lipid profile and platelet aggregation in streptozotocin induced diabetic rats | 2011 | (Asad et al., 2011) |

*Table 4:* Reported literature on antidiabetic activity of *Commiphora wightii (2011-2020)*

| S.No. | Title | Year of publication | Reference |
|---|---|---|---|
| 1. | Pharmacological Uses of the Plants Belonging to the Genus *Commiphora* | 2020 | (Latha et al., 2020) |
| 2. | Guggulipid: A Promising Multi-Purpose Herbal Medicinal Agent | 2020 | (Ahmad et al., 2020) |
| 3. | Googling the Guggul (*Commiphora* and *Boswellia*) for Prevention of Chronic Diseases | 2018 | (Kunnumakkara et al., 2018) |
| 4. | Medicinal plants with traditional use: Ethnobotany in the Indian subcontinent | 2018 | (Rupani & Chavez, |

| | | | |
|---|---|---|---|
| | | | 2018) |
| 6. | Effect of *Commiphora mukul* gum resin on hepatic marker enzymes, lipid peroxidation and antioxidants status in pancreas and heart of streptozotocin induced diabetic rats | 2012 | (Ramesh et al., 2012) |
| 7. | The genus *Commiphora*: a review of its traditional uses, phytochemistry and pharmacology | 2012 | (Shen et al., 2012) |
| 8. | Antihyperglycemic, hypolipidemic and antioxidant activities of ethanolic extract of *Commiphora mukul* gum resin in fructose-fed male Wistar rats | 2012 | (Ramesh & Saralakumari, 2012) |
| 9. | Antihyperglycemic and antioxidant activities of alcoholic extract of *Commiphora mukul* gum resin in streptozotocin induced diabetic rats | 2011 | (Bellamkonda et al., 2011) |

*Table 5: Reported literature on antidiabetic activity on Cyamopis tetragonoloba (2011-2020)*

| **S.No.** | **Title** | **Year of publication** | **Reference** |
|---|---|---|---|
| 1. | Exploring potential new galactomannan source of Retama reatam seeds for food, cosmetic and pharmaceuticals: Characterization and physical, emulsifying and antidiabetic properties | 2019 | (Chouaibi et al., 2019) |
| 2. | Guar gum and its composites as potential materials for diverse applications: A review | 2018 | (Sharma et al., 2018) |
| 3. | Effect of a low dose whey/guar preload on glycemic control in people with type 2 diabetes--a randomised controlled trial | 2014 | (Clifton et al., 2014) |
| 4. | Polyphenols-rich *Cyamopsis tetragonoloba* (L.) Taub. beans show hypoglycemic and β-cells protective effects in type 2 diabetic rats | 2014 | (Gandhi et al., 2014) |

**Herb Drug Interaction (HDI)**

When both herbal medicines and conventional drugs are co-administered then they can lead to clinically relevant HDIs (Gardiner et al., 2006). It occurs when a herbal preparation interferes with the metabolism of the concomitantly administered drug(s) by simply interacting with the enzyme for which it is a substrate and thus causes an alteration in the plasma concenetration of the affected drug (Tarirai et al., 2010). This process has an overall impact on the normal pharmacokinetic profile of the drug. These HDIs are of utmost importance for the drugs with narrow therapeutic windows as their consequences are life threatening (Kennedy et al., 2008).

*Mechanism of HDI*

A single herb contains a number of phytoconstituents (biologically active) which are capable of modulating the physiological actions (which are similar to allopathic drugs). These modulations occur through complex synergistic or antagonistic effects or both of them at once (Mamindla & Koganti, 2016). The process of HDI is mediated by pharmacokinetic (PK) and pharmacodynamic (PD) mechanisms. The former ones are quite difficult to anticipate. Most of the interaction reported till date are PK interactions in which there is a functional modulation of the DMEs (mainly CYPs), DTs (like P-gp). On the other hand, the PD interaction involves the antagonism, addition, synergism and even modulation of the drug targets. The multiple/complex HDIs involved both PK-PD interactions (Fasinu et al., 2012; Zuo et al., 2015).

*1. Pharmacokinetic HDIs*

The PK HDIs can occur at any step of absorption, distribution, metabolism and excretion (ADME).

*Absorption interactions*

Any herb which has an influence on the normal gastrointestinal (GI) tract will definitely have an impact on the absorption pattern of the drug and thus lead to HDI. For instance, the drugs belonging to the class of antacids, which have the potential to increase the pH, will have an effect on the absorption of the herbs with weak acid contents (Katchamart et al., 2000).

*Distribution interactions*

These interactions are shown generally by the drugs having high plasma-binding protein property and a narrow therapeutic window (NTW). For instance, *Agrimonia eupatoria* interfere with the efficacy of anticoagulants (like Warfarin) leading to excessive bleeding or leading to the riskof blood clotting (Borse et al., 2019).

*Metabolism interactions*

Metabolism is defined as the biochemical modifications of the xenobiotics entering into the human body with the help of a special enzymatic system leading to its elimination. The duration and intensity of the pharmacological action of the drug are determined by the rate of metabolism (Ito et al., 2004). A large number of phytochemicals are lipophilic in nature and thus are not easily

eliminated by the body. In such a case, the human body makes them hydrophilic by increasing their reactivity and conjugating them with more polar groups. This is done in two phases in the body (Ito et al., 2004; Zhou et al., 2003). The phase I involves CYP450 enzyme system which oxidizes, reduces or hydrolyze the system and the phase II involves conjugation reactions such as acetylation, sulfation, glucoronidation, etc. (Martignoni et al., 2006). The significance of the individual CYP enzyme in human drug metabolism varies, with CYP3A, CYP2D, and CYP2C being responsible for the metabolism of 50%, 25%, and 20%, respectively, of most of the pharmaceuticals/xenobiotics (Zhang & Tang, 2018). Herbal ingredients can alter metabolizing enzymes through induction and/or inhibition. Induction of CYPs by herbal products usually requires several days; however, induction of the enzyme(s) may lead to decreased drug plasma levels (through increased drug metabolism), and subsequently to reduced drug effects (Lim et al., 2018). Conversely, the inhibition of CYPs is often immediate, and may lead to increased drug plasma levels (through decreased drug metabolism), resulting in an enhanced drug effect, that may result in significant adverse reactions or toxicities.

Mutual competitive inhibition may occur between herbal constituent and a drug, as both are often metabolized by the same CYP isoform. For example, diallyl sulfide from garlic is a competitive inhibitor of CYP2E1 (Teyssier et al., 1999). Noncompetitive inhibition is caused by the binding of herbal constituents containing electrophilic groups (eg, imidazole or hydrazine group) to the heme portion of CYPs. For example, piperine inhibits CYP1A and CYP2A by noncompetitive mechanism. Hyperforin present in St John's wort is also a potent noncompetitive inhibitor of CYP2D6. The mechanism-based inhibition of CYP is due to the formation of a complex between herbal metabolite with CYP under consideration (Dalvi & Dalvi, 1991).

*Elimination interactions*

The sources of drug elimination from the body are urine, feces, sweat, tears, semen, menstrual discharge, etc. The main players in drug or xenobiotic elimination are cell transporter protein/enzymes such as P-glycoprotein (P-gp), organic anion transporting polypeptide (OATP), organic anion transporter, OCTP, breast cancer resistance protein, and others in this process (Zhou et al., 2003). However, these may get affected by concomitant administration of herb and drug resulting in HDIs. Furthermore, some herbs are known diuretic, which can affect the excretion of medicinal drugs (Zhou et al., 2004). The nephrotoxic drug induces kidney damage resulting in a slow rate of elimination leading to an accumulation of herbs and drugs in the body. Important examples of drugs that damage the kidneys include gentamicin, amphotericin B, methotrexate, and tobramycin. Hence, a close monitoring is required to avoid the unwanted HDIs. Furthermore, in case of elimination interactions the role of transporters needs to be focused as transporters govern the movement of xenobiotics in and out of the cells (Zhou et al., 2003).

The mechanism of HDI has been summarized below:

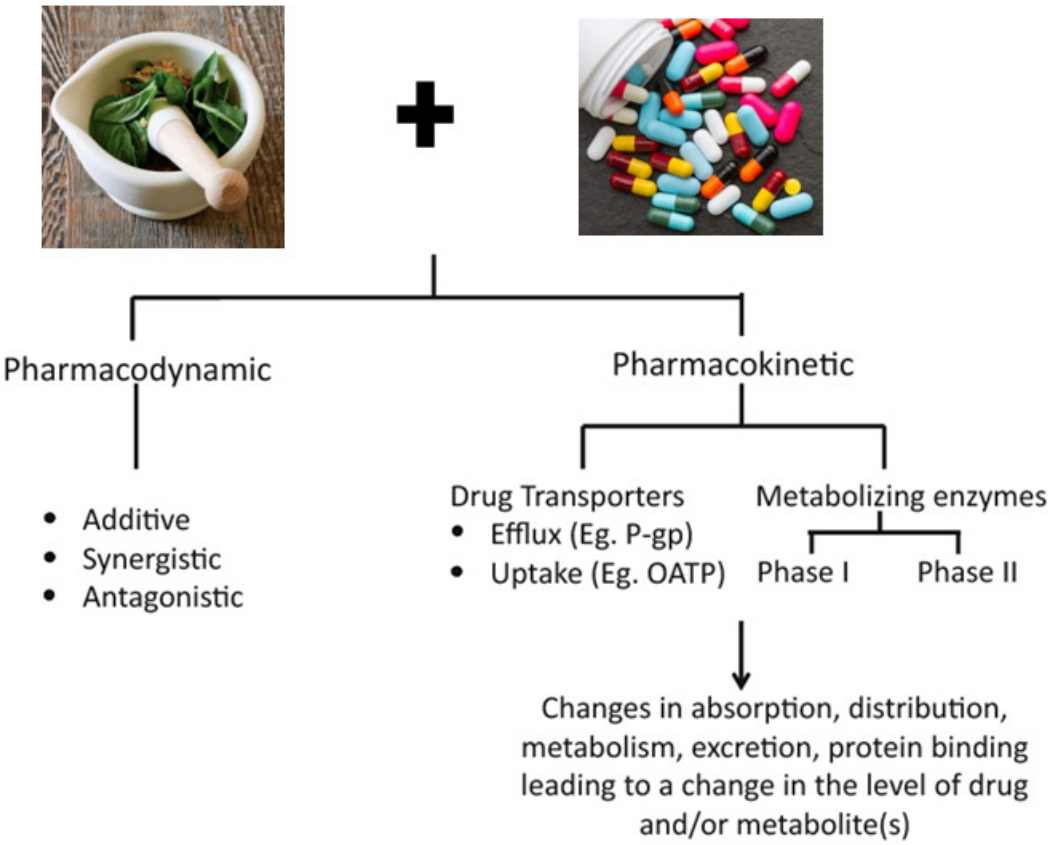



**Fig 4: Mechanism of Herb-Drug Interaction**

*2. Pharmacodynamic HDIs*

Pharmacodynamic interactions are those herb-drug interactions that cause changes in pharmacological responses (e.g., changes in the physiological effect and mechanism of action of the drug on the body and altered relationship of the drug concentration to drug action). These interactions may result in augmentation or inhibition of the pharmacological activity of a co-administered drug. Herb-drug pharmacodynamic interactions would, therefore, involve changes in the pharmacological effects of the drug through additive, synergistic or antagonistic actions (Mamindla & Koganti, 2016; Wojcikowski et al., 2009).

These reactions occur mainly at the receptor level and are classified as direct andindirect HDIs. The direct HDIs are easy to understand and even predict than indirect HDIs. Medication could be of further risk when used with dietary supplements/herbal medicines that share these pharmacological activities (Mamindla & Koganti, 2016). One of the good examples of indirect pharmacodynamic HDIs is cranberry, which potentiate the anticoagulant activity of warfarin. Doubling of the plasma drug concentration may lead to enhanced drug effects and/or adverse effects depending on the

therapeutic and safety window. (Balap et al., 2016). However, less marked changes may still be clinically important for drugs with a steep concentration–response relationship or a narrow therapeutic index (NTI) (Yoo et al., 2018).

*Table 6: Interaction of major phytoconstitutes of the species selected for the study with DMEs( CYPs) and DTs (P-gp)*

| S.No | Phytoconstituent | Interaction with CYPs | Interaction with P-gp | References |
|---|---|---|---|---|
| 1. | Gallic acid | CYP3A4: High Inhibition | High Inhibition | (Vijayakumar et al., 2015) |
| 2. | Ellagic acid | CYP3A4: High Inhibition | High Inhibition | (Athukuri & Neerati, 2017) |
| 3. | Quercetin | CYP3A4: High Inhibition and Inducer<br>CYP2D6: Moderate Inhibition | High Inhibition | (Ekstrand et al., 2015) |
| 4. | β-sitosterol | CYP3A4: Low Inhibition<br>CYP2D6: Low Inhibition | Low Inhibition | (Nair et al., 2007; Vijayakumar et al., 2015) |
| 5. | Guggulsterol, Guggulsterone | CYP3A4: High Induction | High Inhibition | (Kunnumakkara et al., 2018) |
| 6. | Kaempferol | CYP3A4: High Inhibition | High Inhibition | (Marín et al., 2018; Park & Choi, 2019) |
| 7. | Caffeic acid | CYP3A4: Low Inhibition<br>CYP2D6: High Inhibition | High Inhibition | (Rastogi & Jana, 2014; Teng et al., 2020) |

| | | | | |
|---|---|---|---|---|
| 8. | Catechin, Epicatechin, | CYP1A2, 2C9, 2D6, 3A4: Weak Inhibition | High Inhibition | (Satoh et al., 2016) |
| 9. | Epicatechin-3-O-gallate (ECG), Epigallocatechin-3-O-gallate (EGCG) | CYP3A4, 1A2, 2C9: High Inhibition | High Inhibition | (Satoh et al., 2016) |
| 10. | Daidzein | CYP3A4: Both Inhibition and Induction | High Inhibition | (Li et al., 2009; Tanaka et al., 1991) |

**The phytoconstituents discussed above have been reported to alter the metabolism of various co-administerd drugs either by inhibiting or inducing the major DMEs and DTs. Moreover, their co-administration may even augment or inhibit the effect of the allopathic drug. Hence, the prime aim of this research becomes to study the effect of common antidiabetic plant species on the pharmacokinetics and pharmacodynamics of a commonly used oral antidiabetic drug class.**

## Research Gap

From the review of literature discussed above, it can be concluded that these selected species of Rajasthan have not been tested for HDI as there is no available literature for the same. However, the phyto-constituents of these species have already been reported to induce/inhibit the activities of the major DMEs and Pg-P. Hence, there is an urgent need to conduct a detailed research on the HDI using the whole part or the plant parts of these species as they are being used in daily consumption by the people.

## Rationale of the research

As the importance of genomics is rising in the modern healthcare practices and convergence of herbal and precision medicine is the ultimate future of treatment of some common diseases, hence through this research a small step is being taken to study HDI using the DMEs and the DTs. This research will further aid the medical practitioners and people working in the field of Ayurveda to give a more defined Herb-drug combination to the patients.

## Objective of the study

1. To screen the selected anti-diabetic plant species and select one plant species on the basis of their maximum efficacy in terms of antidiabetic effect
2. To study the Herb-drug Interactions among the selected anti-diabetic plant species and known oral antidiabetic drugs (meglinitide analogues).

## Methodology

*Selection of plant species*

As we have selected three plant species for this research, so in the pilot study only one plant species will be selected. The selection will be done on evaluating their antidiabetic efficacy based on the reported literature till date. The plant extract will be prepared by following the standard protocol using suitable solvent (on the basis of the phytochemicals present in the chosen plant specie).

*Experimental animals*

It will be ensured that the animal experimentation protocol is approved by the Institutional Animal Ethics and Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) guidelines will be followed for the use and care of experimental animals in research. Swiss albino mice of either sex will be maintained under standard conditions in an animal house of the institution approved by CPCSEA.

*Cytotoxicity assay*

The cytotoxicity assay will be performed to determine the optimum dose of the herbal extract to be administered in the experimental animal. For this either a dye that selectively stain the dead cells (such as *Trypan Blue)* will be used (Strober, 2015) or the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction assay (Riss et al., 2004) will be performed.

*Antidiabetic study of the plant (using Biochemical Parameters)*

This study will be done to evaluate the pharmacodynamic (additive/ synergistic/ antagonisitic) interactions of the herb with the drug. The antidiabetic potential of plant alone or in combination with the OAD will be evaluated in the Swiss Albino Mice of the either sex. The diabetes will be induced in the mice by administering a single intraperitoneal injection of streptozotocin (STZ) (Soni et al., 2018). For this purpose animal Swiss albino mice will be divided into 5 groups:

*Group I: No treatment will be given*

*Group II: STZ*

The fasting blood glucose level will be measured using the diabetes test-strips (Glucometer) and the mice showing diabetes will only be selected for further study.

*Group III: STZ + Plant extract*

*Group IV: STZ + Drug*

*Group V: STZ + Plant Extract + Drug*

Alongwith the blood glucose level, the levels of total cholesterol, HDL, LDL, triglycerides, creatinine, and uric acid will be measured by using standard protocol. Also, glycosylated hemoglobin (HbA1c) will be estimated using the method of Nayak and Pattabiraman (Nayak & Pattabiraman, 1981). Further, Liver Function Tests (LFTs) will be performed to evaluate levels of various liver enzymes using the standard protocols (Bora et al., 2016).

*Determination of metabolites' level of the drug and the plant extract (in blood plasma)*

On administration of the selected dose of drug and plant/ plant-part extract, their metabolism will occur inside the body. The level of the metabolites in the blood plasma will be measured at two levels:

*In-vitro*: For the *in-vitro* measurement of the metabolites level, *Erythromycin-N-Demethylation Assay* will be conducted in liver microsome as per the protocol mentioned by (Chaudhari et al., 2019).

*In-vivo:* The *in-vivo* measurement of the metabolites' level will be done on the swiss albino mice using LCMS (*Liquid chromatograpgy-mass spectrometry*)/ HPLC (*High performance liquid chromatography*).

*Determination of the levels/activities of DMEs & DTs*

The hepatic/intestinal mRNA or/and protein expression levels of the DMEs and DTs will be measured for the drug and the plant alone or in the combination with the drug using the protocol proposed by (Liu et al., 2016) and enzyme activities will be measured biochemically by standard protocols.

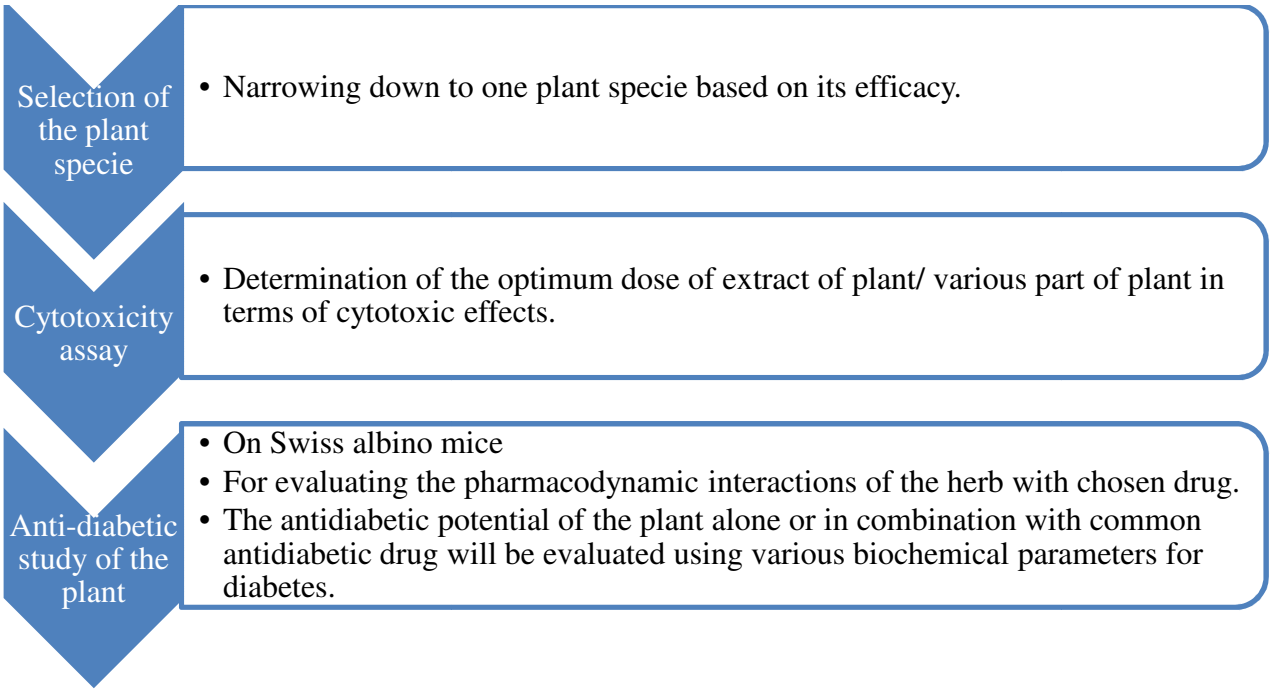
*Pharmakokinetic and statistical analysis*

The pharmacokinetic and statistical analysis will be done using any open source software.

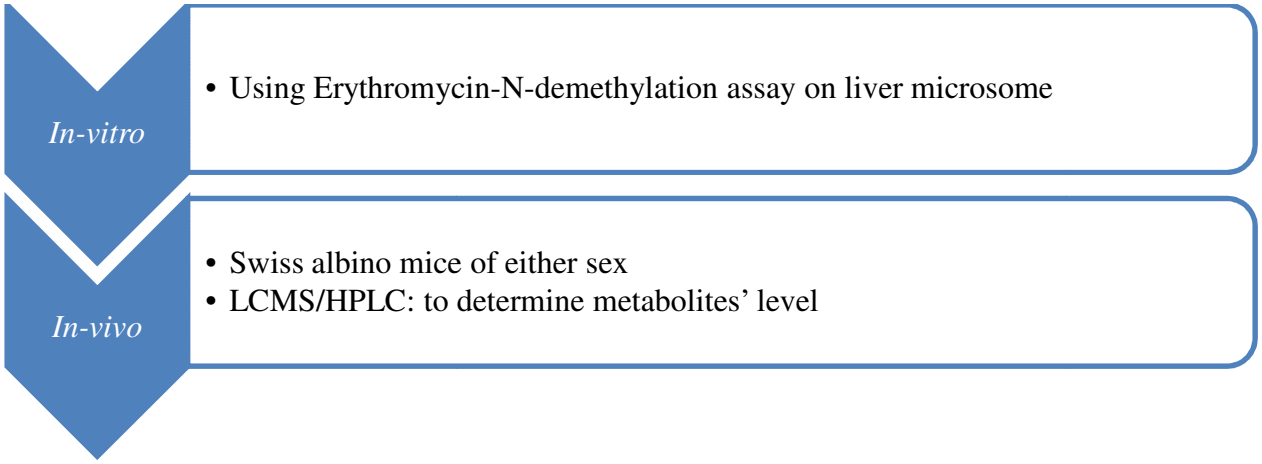
## Plan of work

The research work will be carried out in four phases of 6 months each:

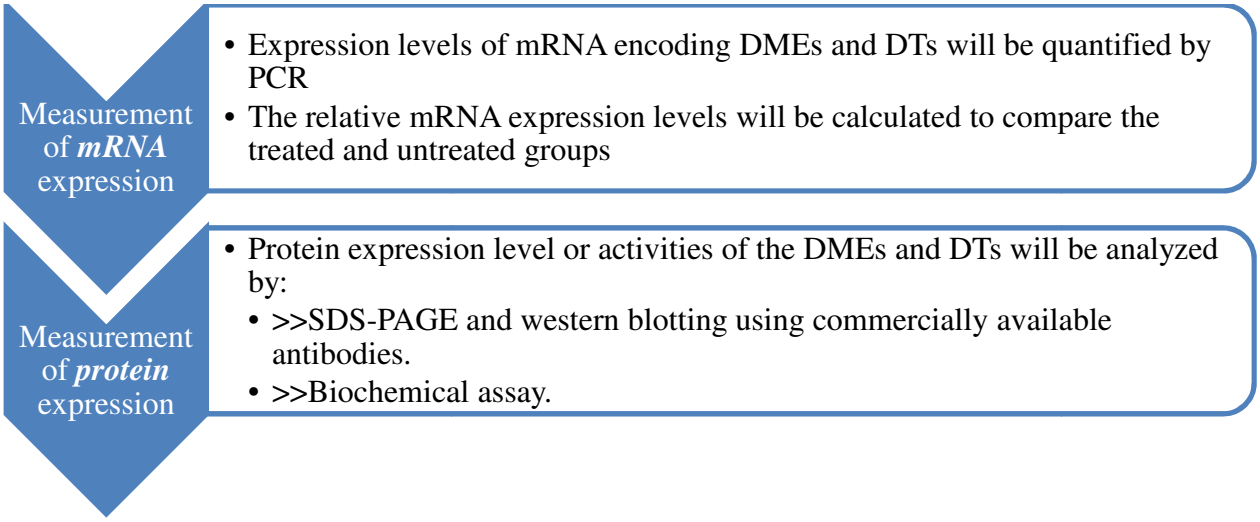
Phase I (6 months): Pilot study


Selection of the plant specie
• Narrowing down to one plant specie based on its efficacy.
Cytotoxicity assay
• Determination of the optimum dose of extract of plant/ various part of plant in terms of cytotoxic effects.
Anti-diabetic study of the plant
• On Swiss albino mice
• For evaluating the pharmacodynamic interactions of the herb with chosen drug.
• The antidiabetic potential of the plant alone or in combination with common antidiabetic drug will be evaluated using various biochemical parameters for diabetes.

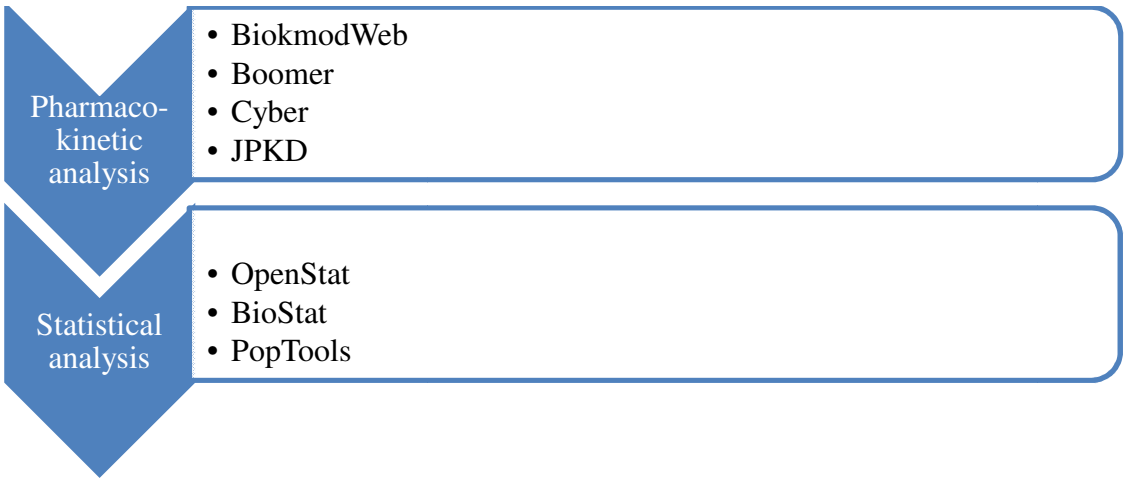
Phase II (6 months): Determination of metabolites' level of the drug and the plant/ plant part extract (in blood plasma)


In-vitro
• Using Erythromycin-N-demethylation assay on liver microsome
In-vivo
• Swiss albino mice of either sex
• LCMS/HPLC: to determine metabolites' level

Phase III (6 months): Determination of the levels/activities of DMEs & DTs of the drug and the plant alone or in combination with the drug


Measurement of *mRNA* expression
• Expression levels of mRNA encoding DMEs and DTs will be quantified by PCR
• The relative mRNA expression levels will be calculated to compare the treated and untreated groups
Measurement of *protein* expression
• Protein expression level or activities of the DMEs and DTs will be analyzed by:
• >>SDS-PAGE and western blotting using commercially available antibodies.
• >>Biochemical assay.

Phase IV (6 months): Checking the hypothesis using any open source pharmacokinetic and statistical software.



## References


Abuelgassim, A. O. (2013). Effect of *Acacia nilotica* fruit extract on serum glucose and lipid concentrations in alloxan-induced diabetic rats. *Pakistan Journal of Biological Sciences: PJBS*, *16*(21), 1398–1402. https://doi.org/10.3923/pjbs.2013.1398.1402

Ahmad, M. A., Mujeeb, M., Akhtar, M., Khushtar, M., Arif, M., & Haque, M. R. (2020). Guggulipid: A Promising Multi-Purpose Herbal Medicinal Agent. *Drug Research*, *70*(4), 123–130. https://doi.org/10.1055/a-1115-4669

American Diabetes Association. (2013). Standards of medical care in diabetes—2013. *Diabetes Care*, *36 Suppl 1*, S11-66. https://doi.org/10.2337/dc13-S011

Anjana, R. M., Deepa, M., Pradeepa, R., Mahanta, J., Narain, K., Das, H. K., Adhikari, P., Rao, P. V., Saboo, B., Kumar, A., Bhansali, A., John, M., Luaia, R., Reang, T., Ningombam, S., Jampa, L., Budnah, R. O., Elangovan, N., Subashini, R., … Yajnik, C. S. (2017). Prevalence of diabetes and prediabetes in 15 states of India: Results from the ICMR–INDIAB population-based cross-sectional study. *The Lancet Diabetes & Endocrinology*, *5*(8), 585–596. https://doi.org/10.1016/S2213-8587(17)30174-2

Anjana, R. M., Pradeepa, R., Deepa, M., Datta, M., Sudha, V., Unnikrishnan, R., Nath, L. M., Das, A. K., Madhu, S. V., Rao, P. V., Shukla, D. K., Kaur, T., Ali, M. K., & Mohan, V. (2011). The Indian Council of Medical Research–India Diabetes (ICMR–INDIAB) Study: Methodological Details. *Journal of Diabetes Science and Technology*, *5*(4), 906–914.

Asad, M., Aslam, M., Munir, T. A., & Nadeem, A. (2011). Effect of *Acacia nilotica* leaves extract on hyperglycaemia, lipid profile and platelet aggregation in streptozotocin induced diabetic rats. *Journal of Ayub Medical College, Abbottabad: JAMC*, *23*(2), 3–7.

Asad, M., Munir, T. A., Farid, S., Aslam, M., & Shah, S. S. (2015). Duration effect of *Acacia nilotica* leaves extract and glibenclamide as hypolipidaemic and hypoglycaemic activity in alloxan

induced diabetic rats. *JPMA. The Journal of the Pakistan Medical Association*, *65*(12), 1266–1270.

Asati, V., & Sharma, P. K. (2019). Purification and characterization of an isoflavones conjugate hydrolyzing β-glucosidase (ICHG) from Cyamopsis tetragonoloba (guar). *Biochemistry and Biophysics Reports*, *20*, 100669. https://doi.org/10.1016/j.bbrep.2019.100669

Athukuri, B. L., & Neerati, P. (2017). Enhanced Oral Bioavailability of Diltiazem by the Influence of Gallic Acid and Ellagic Acid in Male Wistar Rats: Involvement of CYP3A and P-gp Inhibition. *Phytotherapy Research*, *31*(9), 1441–1448. https://doi.org/10.1002/ptr.5873

Balap, A., Atre, B., Lohidasan, S., Sinnathambi, A., & Mahadik, K. (2016). Pharmacokinetic and pharmacodynamic herb-drug interaction of *Andrographis paniculata* (Nees) extract and andrographolide with etoricoxib after oral administration in rats. *Journal of Ethnopharmacology*, *183*, 9–17. https://doi.org/10.1016/j.jep.2015.11.011

Barapatre, A., Aadil, K. R., Tiwary, B. N., & Jha, H. (2015). In vitro antioxidant and antidiabetic activities of biomodified lignin from *Acacia nilotica* wood. *International Journal of Biological Macromolecules*, *75*, 81–89. https://doi.org/10.1016/j.ijbiomac.2015.01.012

Bellamkonda, R., Rasineni, K., Singareddy, S. R., Kasetti, R. B., Pasurla, R., Chippada, A. R., & Desireddy, S. (2011). Antihyperglycemic and antioxidant activities of alcoholic extract of *Commiphora mukul* gum resin in streptozotocin induced diabetic rats. *Pathophysiology: The Official Journal of the International Society for Pathophysiology*, *18*(4), 255–261. https://doi.org/10.1016/j.pathophys.2010.10.002

Black, C., Donnelly, P., McIntyre, L., Royle, P. L., Shepherd, J. P., & Thomas, S. (2007). Meglitinide analogues for type 2 diabetes mellitus. *The Cochrane Database of Systematic Reviews*, *2*, CD004654. https://doi.org/10.1002/14651858.CD004654.pub2

Blicklé, J. (2006). Meglitinide analogues: A review of clinical data focused on recent trials. *Diabetes & Metabolism*, *32*(2), 113–120. https://doi.org/10.1016/S1262-3636(07)70257-4

Bora, K., Borah, M., Chutia, H., Nath, C. K., Das, D., & Ruram, A. A. (2016). Presence of Concurrent Derangements of Liver Function Tests in Type 2 Diabetes and Their Relationship with Glycemic Status: A Retrospective Observational Study from Meghalaya. *Journal of Laboratory Physicians*, *8*(1), 30–35. https://doi.org/10.4103/0974-2727.176227

Borse, S. P., Singh, D. P., & Nivsarkar, M. (2019). Understanding the relevance of herb–drug interaction studies with special focus on interplays: A prerequisite for integrative medicine. *Porto Biomedical Journal*, *4*(2). https://doi.org/10.1016/j.pbj.0000000000000015

Burcelin, R., & Gourdy, P. (2017). Harnessing glucagon-like peptide-1 receptor agonists for the pharmacological treatment of overweight and obesity. *Obesity Reviews: An Official Journal of the International Association for the Study of Obesity*, *18*(1), 86–98. https://doi.org/10.1111/obr.12465

Cariou, B., Charbonnel, B., & Staels, B. (2012). Thiazolidinediones and PPARγ agonists: Time for a reassessment. *Trends in Endocrinology and Metabolism: TEM*, *23*(5), 205–215. https://doi.org/10.1016/j.tem.2012.03.001

Chacko, B. (2019, July 9). *1 In 2 Indian Diabetics Unaware Of Their Condition: Study* |. https://www.indiaspend.com/1-in-2-indian-diabetics-unaware-of-their-condition-study/

Chatterjee, S., Khunti, K., & Davies, M. J. (2017). Type 2 diabetes. *The Lancet*, *389*(10085), 2239–2251. https://doi.org/10.1016/S0140-6736(17)30058-2

Chaudhari, S., Zambad, S., & Ali, M. (2019). Effect of Aqueous Extract of *Azadirachta indica* Leaves on Pharmacokineics and Pharmacodynamics of Glipizide. *Drug Metabolism Letters*, *13*(1), 19–24. https://doi.org/10.2174/1872312812666181106115247

Chaudhury, A., Duvoor, C., Reddy Dendi, V. S., Kraleti, S., Chada, A., Ravilla, R., Marco, A., Shekhawat, N. S., Montales, M. T., Kuriakose, K., Sasapu, A., Beebe, A., Patil, N., Musham, C. K., Lohani, G. P., & Mirza, W. (2017). Clinical Review of Antidiabetic Drugs: Implications for Type 2 Diabetes Mellitus Management. *Frontiers in Endocrinology*, *8*. https://doi.org/10.3389/fendo.2017.00006

Cho, H.-J., & Yoon, I.-S. (2015, January 6). *Pharmacokinetic Interactions of Herbs with Cytochrome P450 and P-Glycoprotein* [Review Article]. Evidence-Based Complementary and Alternative Medicine; Hindawi. https://doi.org/10.1155/2015/736431

Chouaibi, M., Rezig, L., Lakoud, A., Boussaid, A., Hassouna, M., Ferrari, G., & Hamdi, S. (2019). Exploring potential new galactomannan source of *Retama reatam* seeds for food, cosmetic and pharmaceuticals: Characterization and physical, emulsifying and antidiabetic properties. *International Journal of Biological Macromolecules*, *124*, 1167–1176. https://doi.org/10.1016/j.ijbiomac.2018.12.007

Clifton, P. M., Galbraith, C., & Coles, L. (2014). Effect of a low dose whey/guar preload on glycemic control in people with type 2 diabetes—A randomised controlled trial. *Nutrition Journal*, *13*, 103. https://doi.org/10.1186/1475-2891-13-103

Dalvi, R. R., & Dalvi, P. S. (1991). Comparison of the effects of piperine administered intragastrically and intraperitoneally on the liver and liver mixed-function oxidases in rats. *Drug Metabolism and Drug Interactions*, *9*(1), 23–30. https://doi.org/10.1515/dmdi.1991.9.1.23

*Diabetes Mellitus Subtypes*. (2019, June 27). News-Medical.Net. https://www.news-medical.net/health/Diabetes-Mellitus-Subtypes.aspx

Dornhorst, A. (2001). Insulinotropic meglitinide analogues. *The Lancet*, *358*(9294), 1709–1716. https://doi.org/10.1016/S0140-6736(01)06715-0

Ekstrand, B., Rasmussen, M. K., Woll, F., Zlabek, V., & Zamaratskaia, G. (2015, January 21). *In Vitro Gender-Dependent Inhibition of Porcine Cytochrome P450 Activity by Selected Flavonoids and Phenolic Acids* [Research Article]. BioMed Research International; Hindawi. https://doi.org/10.1155/2015/387918

Fasinu, P. S., Bouic, P. J., & Rosenkranz, B. (2012). An Overview of the Evidence and Mechanisms of Herb–Drug Interactions. *Frontiers in Pharmacology*, *3*. https://doi.org/10.3389/fphar.2012.00069

Feingold, K. R. (2000). Oral and Injectable (Non-Insulin) Pharmacological Agents for Type 2 Diabetes. In K. R. Feingold, B. Anawalt, A. Boyce, G. Chrousos, K. Dungan, A. Grossman, J. M. Hershman, G. Kaltsas, C. Koch, P. Kopp, M. Korbonits, R. McLachlan, J. E. Morley, M. New, L. Perreault, J. Purnell, R. Rebar, F. Singer, D. L. Trence, … D. P. Wilson (Eds.), *Endotext*. MDText.com, Inc. http://www.ncbi.nlm.nih.gov/books/NBK279141/

Gandhi, G. R., Vanlalhruaia, P., Stalin, A., Irudayaraj, S. S., Ignacimuthu, S., & Paulraj, M. G. (2014). Polyphenols-rich *Cyamopsis tetragonoloba* (L.) Taub. Beans show hypoglycemic and β-cells protective effects in type 2 diabetic rats. *Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association*, *66*, 358–365. https://doi.org/10.1016/j.fct.2014.02.001

Gardiner, P., Graham, R. E., Legedza, A. T. R., Eisenberg, D. M., & Phillips, R. S. (2006). Factors associated with dietary supplement use among prescription medication users. *Archives of Internal Medicine*, *166*(18), 1968–1974. https://doi.org/10.1001/archinte.166.18.1968

Guardado-Mendoza, R., Prioletta, A., Jiménez-Ceja, L. M., Sosale, A., & Folli, F. (2013). The role of nateglinide and repaglinide, derivatives of meglitinide, in the treatment of type 2 diabetes mellitus. *Archives of Medical Science : AMS*, *9*(5), 936–943. https://doi.org/10.5114/aoms.2013.34991

Hunt, B., Malkin, S. J. P., Moes, R. G. J., Huisman, E. L., Vandebrouck, T., & Wolffenbuttel, B. H. R. (2019). Once-weekly semaglutide for patients with type 2 diabetes: A cost-effectiveness analysis in the Netherlands. *BMJ Open Diabetes Research & Care*, *7*(1), e000705. https://doi.org/10.1136/bmjdrc-2019-000705

Iqbal, M. (2019, December 19). 'High prevalence of pre-diabetes in Rajasthan.' *The Hindu*. https://www.thehindu.com/news/national/other-states/high-prevalence-of-pre-diabetes-in-rajasthan/article30343141.ece

Ito, K., Brown, H. S., & Houston, J. B. (2004). Database analyses for the prediction of *in vivo* drug–drug interactions from in vitro data. *British Journal of Clinical Pharmacology*, *57*(4), 473–486. https://doi.org/10.1111/j.1365-2125.2003.02041.x

Katchamart, S., Stresser, D. M., Dehal, S. S., Kupfer, D., & Williams, D. E. (2000). Concurrent flavin-containing monooxygenase down-regulation and cytochrome P-450 induction by dietary indoles in rat: Implications for drug-drug interaction. *Drug Metabolism and Disposition: The Biological Fate of Chemicals*, *28*(8), 930–936.

Kennedy, J., Wang, C.-C., & Wu, C.-H. (2008). Patient Disclosure about Herb and Supplement Use among Adults in the US. *Evidence-Based Complementary and Alternative Medicine : ECAM*, *5*(4), 451–456. https://doi.org/10.1093/ecam/nem045

Kim, S.-B., Yoon, I.-S., Kim, K.-S., Cho, S.-J., Kim, Y. S., Cho, H.-J., Chung, S.-J., Chong, S., & Kim, D.-D. (2014). In vitro and in vivo evaluation of the effect of puerarin on hepatic cytochrome p450-mediated drug metabolism. *Planta Medica*, *80*(7), 561–567. https://doi.org/10.1055/s-0034-1368350

Kunnumakkara, A. B., Banik, K., Bordoloi, D., Harsha, C., Sailo, B. L., Padmavathi, G., Roy, N. K., Gupta, S. C., & Aggarwal, B. B. (2018). Googling the Guggul (*Commiphora* and *Boswellia*)

for Prevention of Chronic Diseases. *Frontiers in Pharmacology*, *9*. https://doi.org/10.3389/fphar.2018.00686

Landgraf, R. (2000). Meglitinide Analogues in the Treatment of Type 2 Diabetes Mellitus. *Drugs & Aging*, *17*(5), 411–425. https://doi.org/10.2165/00002512-200017050-00007

Latha, S., Selvamani, P., & Prabha, T. (2020). Pharmacological Uses of the Plants Belonging to the Genus *Commiphora*. *Cardiovascular & Hematological Agents in Medicinal Chemistry*. https://doi.org/10.2174/1871525718666200702125558

Li, Y., Ross-Viola, J. S., Shay, N. F., Moore, D. D., & Ricketts, M.-L. (2009). Human CYP3A4 and murine Cyp3A11 are regulated by equol and genistein via the pregnane X receptor in a species-specific manner. *The Journal of Nutrition*, *139*(5), 898–904. https://doi.org/10.3945/jn.108.103572

Lim, J. W., Chee, S. X., Wong, W. J., He, Q. L., & Lau, T. C. (2018). Traditional Chinese medicine: Herb-drug interactions with aspirin. *Singapore Medical Journal*, *59*(5), 230–239. https://doi.org/10.11622/smedj.2018051

Liu, Y., Luo, X., Yang, C., Yang, T., Zhou, J., & Shi, S. (2016). Impact of quercetin-induced changes in drug-metabolizing enzyme and transporter expression on the pharmacokinetics of cyclosporine in rats. *Molecular Medicine Reports*, *14*(4), 3073–3085. https://doi.org/10.3892/mmr.2016.5616

Mamindla, S., & Koganti, B. (2016). *HERB-DRUG INTERACTIONS: AN OVERVIEW OF MECHANISMS AND CLINICAL ASPECTS*. /paper/HERB-DRUG-INTERACTIONS%3A-AN-OVERVIEW-OF-MECHANISMS-Mamindla-Koganti/5962a7aa10b581e28842f0d6fb865abf635e1db4

Marín, L., Gutiérrez-del-Río, I., Entrialgo-Cadierno, R., Villar, C. J., & Lombó, F. (2018). *De novo* biosynthesis of myricetin, kaempferol and quercetin in *Streptomyces albus* and *Streptomyces coelicolor*. *PLOS ONE*, *13*(11), e0207278. https://doi.org/10.1371/journal.pone.0207278

Martignoni, M., Groothuis, G. M. M., & de Kanter, R. (2006). Species differences between mouse, rat, dog, monkey and human CYP-mediated drug metabolism, inhibition and induction. *Expert Opinion on Drug Metabolism & Toxicology*, *2*(6), 875–894. https://doi.org/10.1517/17425255.2.6.875

Meena, P. D., Kaushik, P., Shukla, S., Soni, A. K., Kumar, M., & Kumar, A. (2006). Anticancer and antimutagenic properties of *Acacia nilotica* (Linn.) on 7,12-dimethylbenz(a)anthracene-induced skin papillomagenesis in Swiss albino mice. *Asian Pacific Journal of Cancer Prevention: APJCP*, *7*(4), 627–632.

Meijerman, I., Beijnen, J. H., & Schellens, J. H. M. (2006). Herb-drug interactions in oncology: Focus on mechanisms of induction. *The Oncologist*, *11*(7), 742–752. https://doi.org/10.1634/theoncologist.11-7-742

Mohan, V., Anjana, R. M., Pradeepa, R., Unnikrishnan, R., Tanvir, K., & Das, A. K. (2017). The ICMR INDIAB Study – A Compendium of Type 2 Diabetes in India: Lessons Learnt for the Nation. In A. Muruganathan & B. R. Bansode (Eds.), *Progress in Medicine 2017* (p. 1104). API. http://mdrf-eprints.in/1057/

Nair, V. D. P., Foster, B. C., Thor Arnason, J., Mills, E. J., & Kanfer, I. (2007). *In vitro* evaluation of human cytochrome P450 and P-glycoprotein-mediated metabolism of some phytochemicals in extracts and formulations of African potato. *Phytomedicine: International Journal of Phytotherapy and Phytopharmacology*, *14*(7–8), 498–507. https://doi.org/10.1016/j.phymed.2006.12.004

Nayak, S. S., & Pattabiraman, T. N. (1981). A new colorimetric method for the estimation of glycosylated hemoglobin. *Clinica Chimica Acta; International Journal of Clinical Chemistry*, *109*(3), 267–274. https://doi.org/10.1016/0009-8981(81)90312-0

Olokoba, A. B., Obateru, O. A., & Olokoba, L. B. (2012). Type 2 Diabetes Mellitus: A Review of Current Trends. *Oman Medical Journal*, *27*(4), 269–273. https://doi.org/10.5001/omj.2012.68

Omara, E. A., Nada, S. A., Farrag, A. R. H., Sharaf, W. M., & El-Toumy, S. A. (2012). Therapeutic effect of *Acacia nilotica* pods extract on streptozotocin induced diabetic nephropathy in rat. *Phytomedicine: International Journal of Phytotherapy and Phytopharmacology*, *19*(12), 1059–1067. https://doi.org/10.1016/j.phymed.2012.07.006

Overbeeke, N., Termorshuizen, G. H., Giuseppin, M. L., Underwood, D. R., & Verrips, C. T. (1990). Secretion of the alpha-galactosidase from *Cyamopsis tetragonoloba* (guar) by *Bacillus subtilis*. *Applied and Environmental Microbiology*, *56*(5), 1429–1434.

Pakkir Maideen, N. M., Manavalan, G., & Balasubramanian, K. (2018). Drug interactions of meglitinide antidiabetics involving CYP enzymes and OATP1B1 transporter. *Therapeutic Advances in Endocrinology and Metabolism*, *9*(8), 259–268. https://doi.org/10.1177/2042018818767220

Pandey, M. M., Rastogi, S., & Rawat, A. K. S. (2013, June 23). *Indian Traditional Ayurvedic System of Medicine and Nutritional Supplementation* [Review Article]. Evidence-Based Complementary and Alternative Medicine; Hindawi. https://doi.org/10.1155/2013/376327

Park, J.-W., & Choi, J.-S. (2019). Role of kaempferol to increase bioavailability and pharmacokinetics of nifedipine in rats. *Chinese Journal of Natural Medicines*, *17*(9), 690–697. https://doi.org/10.1016/S1875-5364(19)30083-4

Ramachandran, A. (2014). Know the signs and symptoms of diabetes. *The Indian Journal of Medical Research*, *140*(5), 579–581.

Ramesh, B., Karuna, R., Sreenivasa, R. S., Haritha, K., Sai, M. D., Sasi, B. R. B., & Saralakumari, D. (2012). Effect of *Commiphora mukul* gum resin on hepatic marker enzymes, lipid peroxidation and antioxidants status in pancreas and heart of streptozotocin induced diabetic rats. *Asian Pacific Journal of Tropical Biomedicine*, *2*(11), 895–900. https://doi.org/10.1016/S2221-1691(12)60249-4

Ramesh, B., & Saralakumari, D. (2012). Antihyperglycemic, hypolipidemic and antioxidant activities of ethanolic extract of *Commiphora mukul* gum resin in fructose-fed male Wistar rats. *Journal of Physiology and Biochemistry*, *68*(4), 573–582. https://doi.org/10.1007/s13105-012-0175-x

Rastogi, H., & Jana, S. (2014). Evaluation of Inhibitory Effects of Caffeic acid and Quercetin on Human Liver Cytochrome P450 Activities. *Phytotherapy Research*, *28*(12), 1873–1878. https://doi.org/10.1002/ptr.5220

Rehani, P. R., Iftikhar, H., Nakajima, M., Tanaka, T., Jabbar, Z., & Rehani, R. N. (2019). Safety and Mode of Action of Diabetes Medications in comparison with 5-Aminolevulinic Acid (5-ALA). *Journal of Diabetes Research*, *2019*. https://doi.org/10.1155/2019/4267357

Rena, G., Hardie, D. G., & Pearson, E. R. (2017). The mechanisms of action of metformin. *Diabetologia*, *60*(9), 1577–1585. https://doi.org/10.1007/s00125-017-4342-z

Riss, T. L., Moravec, R. A., Niles, A. L., Duellman, S., Benink, H. A., Worzella, T. J., & Minor, L. (2004). Cell Viability Assays. In G. S. Sittampalam, A. Grossman, K. Brimacombe, M. Arkin, D. Auld, C. P. Austin, J. Baell, B. Bejcek, J. M. M. Caaveiro, T. D. Y. Chung, N. P. Coussens, J. L. Dahlin, V. Devanaryan, T. L. Foley, M. Glicksman, M. D. Hall, J. V. Haas, S. R. J. Hoare, J. Inglese, … X. Xu (Eds.), *Assay Guidance Manual*. Eli Lilly & Company and the National Center for Advancing Translational Sciences. http://www.ncbi.nlm.nih.gov/books/NBK144065/

Roozbeh, N., Darvish, L., & Abdi, F. (2017). Hypoglycemic effects of *Acacia nilotica* in type II diabetes: A research proposal. *BMC Research Notes*, *10*(1), 331. https://doi.org/10.1186/s13104-017-2646-1

Rupani, R., & Chavez, A. (2018). Medicinal plants with traditional use: Ethnobotany in the Indian subcontinent. *Clinics in Dermatology*, *36*(3), 306–309. https://doi.org/10.1016/j.clindermatol.2018.03.005

Saha, M. R., Dey, P., Sarkar, I., De Sarker, D., Haldar, B., Chaudhuri, T. K., & Sen, A. (2018). *Acacia nilotica* leaf improves insulin resistance and hyperglycemia associated acute hepatic injury and nephrotoxicity by improving systemic antioxidant status in diabetic mice. *Journal of Ethnopharmacology*, *210*, 275–286. https://doi.org/10.1016/j.jep.2017.08.036

Sarup, P., Bala, S., & Kamboj, S. (2015, October 26). *Pharmacology and Phytochemistry of Oleo-Gum Resin of Commiphora wightii (Guggulu)* [Review Article]. Scientifica; Hindawi. https://doi.org/10.1155/2015/138039

Satoh, T., Fujisawa, H., Nakamura, A., Takahashi, N., & Watanabe, K. (2016). Inhibitory Effects of Eight Green Tea Catechins on Cytochrome P450 1A2, 2C9, 2D6, and 3A4 Activities. *Journal of Pharmacy & Pharmaceutical Sciences: A Publication of the Canadian Society for Pharmaceutical Sciences, Societe Canadienne Des Sciences Pharmaceutiques*, *19*(2), 188–197. https://doi.org/10.18433/J3MS5C

Shah, R., Gulati, V., & Palombo, E. A. (2012). Pharmacological properties of guggulsterones, the major active components of gum guggul. *Phytotherapy Research: PTR*, *26*(11), 1594–1605. https://doi.org/10.1002/ptr.4647

Sharma, G., Sharma, S., Kumar, A., Al-Muhtaseb, A. H., Naushad, M., Ghfar, A. A., Mola, G. T., & Stadler, F. J. (2018). Guar gum and its composites as potential materials for diverse

applications: A review. *Carbohydrate Polymers*, *199*, 534–545. https://doi.org/10.1016/j.carbpol.2018.07.053

Shekar, C., Nagarajappa, R., Singh, R., & Thakur, R. (2015). Antimicrobial efficacy of *Acacia nilotica, Murraya koenigii L. Sprengel, Eucalyptus hybrid,* and *Psidium guajava* on primary plaque colonizers: An in vitro comparison between hot and cold extraction process. *Journal of Indian Society of Periodontology*, *19*(2), 174–179. https://doi.org/10.4103/0972-124X.145814

Shen, T., Li, G.-H., Wang, X.-N., & Lou, H.-X. (2012). The genus *Commiphora*: A review of its traditional uses, phytochemistry and pharmacology. *Journal of Ethnopharmacology*, *142*(2), 319–330. https://doi.org/10.1016/j.jep.2012.05.025

Sivaprasad, S., Raman, R., Conroy, D., Mohan, Wittenberg, R., Rajalakshmi, R., Majeed, A., Krishnakumar, S., Prevost, T., Parameswaran, S., Turowski, P., Maheswari, U., Khobragade, R., Netuveli, G., Sadanandan, R., Greenwood, J., Ramasamy, K., Rao, M., Bergeles, C., & Das, T. (2020). The ORNATE India Project: United Kingdom–India Research Collaboration to tackle visual impairment due to diabetic retinopathy. *Eye*, *34*(7), 1279–1286. https://doi.org/10.1038/s41433-020-0854-8

Sola, D., Rossi, L., Schianca, G. P. C., Maffioli, P., Bigliocca, M., Mella, R., Corlianò, F., Fra, G. P., Bartoli, E., & Derosa, G. (2015). Sulfonylureas and their use in clinical practice. *Archives of Medical Science : AMS*, *11*(4), 840–848. https://doi.org/10.5114/aoms.2015.53304

Song, R. (2016). Mechanism of Metformin: A Tale of Two Sites. *Diabetes Care*, *39*(2), 187–189. https://doi.org/10.2337/dci15-0013

Soni, L. K., Dobhal, M. P., Arya, D., Bhagour, K., Parasher, P., & Gupta, R. S. (2018). *In vitro* and *in vivo* antidiabetic activity of isolated fraction of *Prosopis cineraria* against streptozotocin-induced experimental diabetes: A mechanistic study. *Biomedicine & Pharmacotherapy* =

*Biomedecine & Pharmacotherapie*, *108*, 1015–1021. https://doi.org/10.1016/j.biopha.2018.09.099

Strober, W. (2015). Trypan Blue Exclusion Test of Cell Viability. *Current Protocols in Immunology*, *111*, A3.B.1-A3.B.3. https://doi.org/10.1002/0471142735.ima03bs111

Tanaka, K., Teruo, T., Sasaki, K., Utsunomiya, H., Tanaka, S., Shoji, T., & Hayakawa, H. (1991). Medical vs surgical treatment of acute aortic dissection in an intensive care unit. *Japanese Circulation Journal*, *55*(8), 815–820. https://doi.org/10.1253/jcj.55.815

Tarirai, C., Viljoen, A. M., & Hamman, J. H. (2010). Herb-drug pharmacokinetic interactions reviewed. *Expert Opinion on Drug Metabolism & Toxicology*, *6*(12), 1515–1538. https://doi.org/10.1517/17425255.2010.529129

Teng, Y.-N., Wang, C. C. N., Liao, W.-C., Lan, Y.-H., & Hung, C.-C. (2020). Caffeic Acid Attenuates Multi-Drug Resistance in Cancer Cells by Inhibiting Efflux Function of Human P-glycoprotein. *Molecules (Basel, Switzerland)*, *25*(2). https://doi.org/10.3390/molecules25020247

Teyssier, C., Guenot, L., Suschetet, M., & Siess, M. H. (1999). Metabolism of diallyl disulfide by human liver microsomal cytochromes P-450 and flavin-containing monooxygenases. *Drug Metabolism and Disposition: The Biological Fate of Chemicals*, *27*(7), 835–841.

Thornberry, N. A., & Gallwitz, B. (2009). Mechanism of action of inhibitors of dipeptidyl-peptidase-4 (DPP-4). *Best Practice & Research. Clinical Endocrinology & Metabolism*, *23*(4), 479–486. https://doi.org/10.1016/j.beem.2009.03.004

Trikkalinou, A., Papazafiropoulou, A. K., & Melidonis, A. (2017). Type 2 diabetes and quality of life. *World Journal of Diabetes*, *8*(4), 120–129. https://doi.org/10.4239/wjd.v8.i4.120

Tripp, M. L., Darland, G., Konda, V. R., Pacioretty, L. M., Chang, J.-L., Bland, J. S., & Babish, J. G. (2012). Optimized mixture of hops rho iso-alpha acids-rich extract and acacia

proanthocyanidins-rich extract reduces insulin resistance in 3T3-L1 adipocytes and improves glucose and insulin control in db/db mice. *Nutrition Research and Practice*, *6*(5), 405–413. https://doi.org/10.4162/nrp.2012.6.5.405

Vella, A. (2012). Mechanism of Action of DPP-4 Inhibitors—New Insights. *The Journal of Clinical Endocrinology and Metabolism*, *97*(8), 2626–2628. https://doi.org/10.1210/jc.2012-2396

Vijayakumar, T. M., Kumar, R. M., Agrawal, A., Dubey, G. P., & Ilango, K. (2015). Comparative inhibitory potential of selected dietary bioactive polyphenols, phytosterols on CYP3A4 and CYP2D6 with fluorometric high-throughput screening. *Journal of Food Science and Technology*, *52*(7), 4537–4543. https://doi.org/10.1007/s13197-014-1472-x

Wojcikowski, K., Wohlmuth, H., Johnson, D. W., Rolfe, M., & Gobe, G. (2009). An *in vitro* investigation of herbs traditionally used for kidney and urinary system disorders: Potential therapeutic and toxic effects. *Nephrology (Carlton, Vic.)*, *14*(1), 70–79. https://doi.org/10.1111/j.1440-1797.2008.01017.x

Yoo, J.-H., Yim, S.-V., & Lee, B.-C. (2018, January 23). *Study of Pharmacodynamic and Pharmacokinetic Interaction of Bojungikki-Tang with Aspirin in Healthy Subjects and Ischemic Stroke Patients* [Research Article]. Evidence-Based Complementary and Alternative Medicine; Hindawi. https://doi.org/10.1155/2018/9727240

Zhang, Z., & Tang, W. (2018). Drug metabolism in drug discovery and development. *Acta Pharmaceutica Sinica B*, *8*(5), 721–732. https://doi.org/10.1016/j.apsb.2018.04.003

Zhou, S., Gao, Y., Jiang, W., Huang, M., Xu, A., & Paxton, J. W. (2003). Interactions of herbs with cytochrome P450. *Drug Metabolism Reviews*, *35*(1), 35–98. https://doi.org/10.1081/dmr-120018248

Zhou, S., Lim, L. Y., & Chowbay, B. (2004). Herbal modulation of P-glycoprotein. *Drug Metabolism Reviews*, *36*(1), 57–104. https://doi.org/10.1081/dmr-120028427

Zuo, Z., Huang, M., Kanfer, I., Chow, M. S. S., & Cho, W. C. S. (2015, February 22). *Herb-Drug Interactions: Systematic Review, Mechanisms, and Therapies* [Editorial]. Evidence-Based Complementary and Alternative Medicine; Hindawi. https://doi.org/10.1155/2015/239150